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Penicillins as β-lactamase-dependent prodrugs: enabling role of a vinyl ester exocyclic to the lactam ring†

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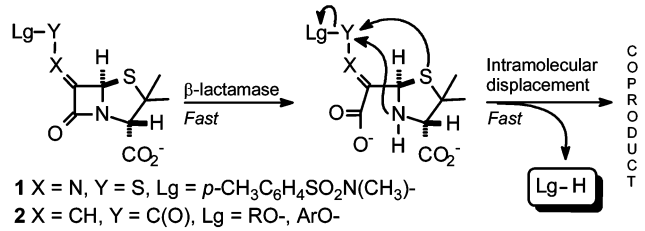
Incorporation of a vinyl ester exocyclic to the β-lactam ring of a penicillin nucleus enables this to act as a β-lactamase-dependent prodrug – rapid release of the (unactivated) alkoxy component of the vinyl ester is triggered by enzyme-catalysed hydrolysis of the β-lactam ring, whilst buffer-catalysed hydrolysis of the structure at neutral pH is particularly slow.

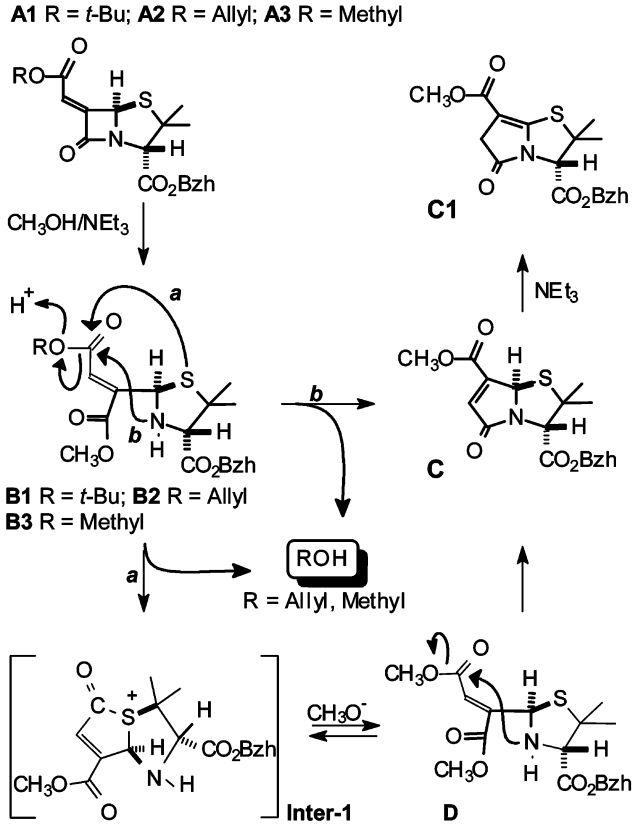
Enzyme-catalysed scission of a β-lactam ring can provide discrete activation of prodrugs based on penicillin, cephalosporin and closely related structures. Elimination of the 3′-substituent of cephems and the 2′-substituent of penems is an inbuilt feature, whereas, a structural modification is required to engender an appropriate reactivity pattern in penams. Incorporation of an unsaturated linker at the 6-position, that has a moderately nucleofugic group attached *via* an electron deficient centre, has been shown to provide such a pattern. The prototypic structure was the *S*-aminosulfeniminopenicillin **1** (Scheme 1) for which it was found that rapid loss of the sulfur-attached moiety (*N*-methyl-*p*-toluenesulfonamide) occurred consequent on scission of the β-lactam ring.[1] The efficacy of this displacement is due to the rapidity of an intramolecular nucleophilic substitution occurring within an acyclic five-membered unit of restricted conformational space (due to the unsaturated linker). Herein, we report that a vinyl ester bearing a simple alcohol as the displaceable moiety, **2** (Scheme 1), is a viable alternative to the *S*-aminosulfenimine construct. This finding augments the chemistry of the penam structure and extends the scope of this to be configured as β-lactamase-dependent prodrugs.

The penicillin structures **A1**–**A3** (*Z*-isomers) were prepared by the method of Buynak,[2] and the behaviour of these in weakly basic methanol was examined in terms of the reaction patterns shown in Scheme 2. The question of interest was to what extent displacement of the alcohol moiety from the vinyl ester would be influenced by scission of the β-lactam ring? The *t*-butyl derivative **A1** was quantitatively converted to the ring-cleaved structure **B1** within 1 h at room temperature with ~0.2 M NEt_3. In contrast, both the allyl and methyl derivatives **A2** and **A3** were completely converted into the ring-fused γ-lactam **C1** and the corresponding alcohol after 40 min under the same reaction conditions.[3] In the case of **A2**, a sample isolated after a 15 min reaction time (using ~0.02 M NEt_3) was found to contain **A2** (~50%), ring-cleaved structure **B2** (~37%) and γ-lactam **C1** (~13%). It is evident that conversion of the ring-cleaved structures **B2/B3** to a γ-lactam is a facile reaction[4] and, as neither allyl alcohol nor methanol is an activated leaving group, the driving force lies in the kinetic and thermodynamic aptness of these ring-cleaved structures to undergo an intramolecular nucleophilic displacement.[5] Two reaction paths, distinguished by the identity of the kinetically dominant nucleophile, are shown for this in Scheme 2. In the case of the *S*-aminosulfeniminopenicillin, stereochemical data of the co-product formed in a CH_3OH/NEt_3 reaction system, identified the thiazolidine-ring sulfur as the kinetically dominant nucleophile whilst the thermodynamically stable end product involved the thiazolidine-ring nitrogen.[1a,b] In the present work this issue was resolved in a study of the hydrolysis of the salt **A3′** (Scheme 3).

Progress of the hydrolysis in D_2O buffer (0.2 M phosphate, pD 7.2, 18 °C) was monitored by ^1H NMR spectroscopy. Changes in the chemical shifts of the methyl ester singlet (3.81 in **A3′** to 3.35 ppm in CH_3OH) and of the thiazolidine-ring H-3 singlet (4.37 in **A3′** to 4.20 ppm in the co-product) were diagnostic features. After 120 h in this buffer the hydrolysis had progressed ~30% with the balance of the material being intact **A3′**. When a portion of β-lactamase enzyme sufficient to generate immediate hydrolysis of the β-lactam ring[6] was added to a sample of **A3′** in this buffer, the



Scheme 1



Scheme 2

† Electronic supplementary information (ESI) available: experimental details of the syntheses and characterisation data on all structures. See http://www.rsc.org/suppdata/cc/b4/b409517k/

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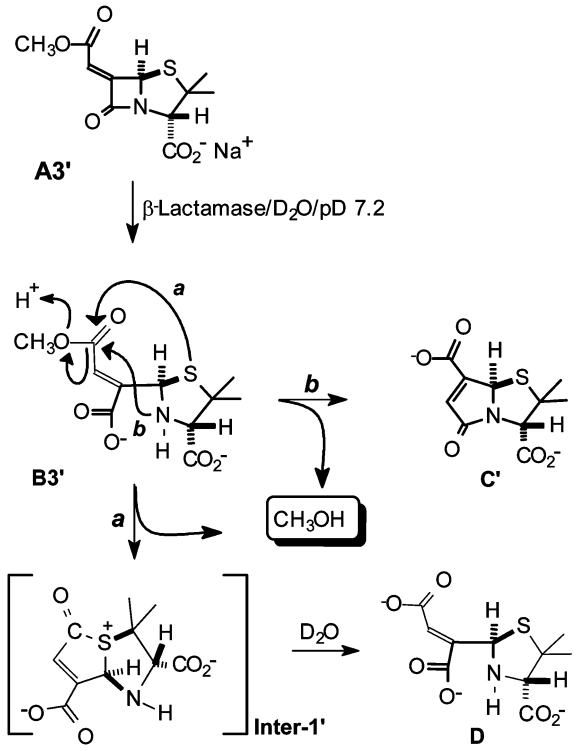
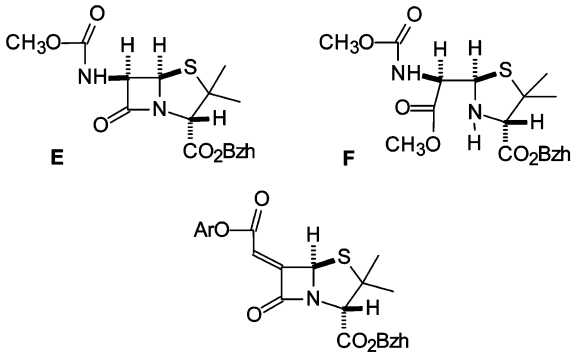


Scheme 3

liberation of methanol and formation of the co-product were complete in less than 20 min. The co-product that was generated in the buffer- and enzyme-catalysed processes was identical; the ^{1}H, ^{13}C NMR and HRMS data† of this material support its identification as the γ-lactam **C**′. Thus, the kinetically dominant nucleophile toward a carbonyl group is the thiazolidine-ring nitrogen whilst this role falls to the thiazolidine-ring sulfur in the the ***S***-aminosulfenimine which has a sulfur atom as the electron-deficient centre.

The role of the unsaturated linker in facilitating the intramolecular displacement within the ring-cleaved structure was assessed by studying carbamate **E**. This was completely converted to the ring-opened structure **F** after 45 min in methanol containing ~0.2 M NEt_3; no ring-fused imidazolidinone structure[7] was formed. It is clear that the unsaturated linker is essential for rapid release of the simple alkoxy component of the ester in the β-lactam-ring-cleaved structures such as **B3**′.



A4 ArOH = 7-hydroxy-4-methylcoumarin

Variation of the displaceable moiety was achieved by selective removal ($Pd(PPh_3)_4$) of the allyl group of **A2** followed by DCC mediated coupling of the resulting free acid with 7-hydroxy-4-methylcoumarin to give the (non-fluorescent) ester **A4**. Treatment of **A4** with CH_3OH/NEt_3, as described above, led to the rapid release of 7-hydroxy-4-methylcoumarin (as a fluorescent component) with the concomitant formation of γ-lactam **C1**.

In conclusion, we have shown that incorporation of a vinyl ester exocyclic to the β-lactam ring of a penicillin nucleus enables this to act as a β-lactamase-dependent prodrug – rapid release of the (unactivated) alkoxy component of the vinyl ester is triggered by enzyme-catalysed hydrolysis of the β-lactam ring. The vinyl ester sidechain is more amenable to synthetic variation than the *S*-aminosulfenimine unit, with the result that the scope to configure penams as β-lactamase-dependent prodrugs is extended considerably. Potential applications of such materials arise in combating certain forms of antibiotic resistance (*via* release of an antimicrobial entity – *e.g.* triclosan[8] – on contact with a β-lactamase enzyme), in the ADEPT mode of drug targeting[9] (*e.g. via* site-specific release and activation of DNA-minor-groove alkylating agents related to Carzelesin[10]), and in the development of chromogenic and fluorogenic substrates[11] as diagnostics for certain classes of β-lactamase enzymes.

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3 Structure **C1** was shown by semiempirical modelling (AM1) to be approximately 14 kcal mol^{-1} more stable than its isomeric form **C**.

4 In the case of **B1**, steric hindrance by the *t*-butyl group blocks addition of the nucleophile to the ester carbonyl, which is the rate-determining step.

5 A parallel for this is found in the intramolecular nucleophilic addition that occurs within the β-lactam-ring-cleaved structure derived from 6(*Z*)-acetylmethylenepenicillanic acid (a potent β-lactamase inhibitor): M. Arisawa and S. Adam, *Biochem. J.*, 1983, **211**, 447–454.

6 6(*Z*)-*t*-butoxycarbonylmethylenepenicillanate and 6(*Z*)-methoxycarbonylmethylenepenicillanate have been found to be β-lactamase substrates (the corresponding sulfones are inhibitors), however, the fate of the β-lactam-ring-cleaved structures was not reported on: (*a*) J. D. Buynak, B. Geng, B. Bachmann and L. Hua, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 1513–1518; (*b*) D. Habich and K. Metzger, *Heterocycles*, 1986, **24**, 289–296.

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